

A Chemical Fractionation for Structure–Function Relations of Soil Organic Matter in Nutrient Cycling

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ABSTRACT

Chemical extractions of soil organic matter (SOM) have not been widely used to elucidate the dynamics of SOM in field settings, especially to address issues of nutrient cycling. To illustrate potential applications of chemical extractions to nutrient issues, this report reviews studies in which the extraction of SOM fractions was based on their binding to polyvalent soil cations. Radiocarbon ages and cycling rates of ^{13}C and ^{15}N indicated that the unbound mobile humic acid (MHA) fraction cycled faster than did the cationic-bound calcium humate (CaHA) fraction. Analyses for C, N, H, and O concentrations and for biochemical groups including carboxyl, phenol, amino, diester P, and free radicals demonstrated that the MHA consisted of more labile and less humified materials than did the CaHA. Quantities and chemical natures of both fractions responded to recent crop management, especially those of the MHA. Three case studies are described in which characterization of the MHA and CaHA contributed toward a process-level understanding of nutrient cycling: (i) a phenol accumulation in the MHA fraction was linked to an inhibition of N mineralization in tropical lowland soils under continuous rice (*Oryza sativa* L.) cropping, (ii) addition of the MHA to California cotton (*Gossypium hirsutum* L.) soils in laboratory studies resulted in increased K availability and plant K uptake, reproducing the benefit of animal manure application in field conditions, and (iii) effects of straw management and winter flooding on N cycling in California rice soils were elucidated by studying a fraction comparable to the MHA fraction. This fractionation is well suited for studying N dynamics, especially in soils enriched in phenolic compounds, and it enabled the linkage of SOM function with chemical nature. It worked well in C-rich flooded soils but needs further evaluation in upland aerobic soils. Further insight into chemical structure and function relations might be achieved by its integration with physical and biological extractions.

IN THE SURFACE LAYER of most soils, SOM contains >90% of the total N and S and up to 75% of the total P (Stevenson, 1994). The chemical nature of SOM is thought to influence the mineralization of these organic nutrients into plant-available forms because some chemical compounds are considered more resistant to microbial degradation than others (Stevenson and Cole, 1999). Few SOM studies, though, have demonstrated such a chemical structure–function relation for a range of soil types.

Progress toward this goal has been slowed by the fact that the most advanced spectroscopic analyses for the chemical structure of SOM, especially nuclear magnetic resonance (NMR) spectroscopy, are best suited to purely organic samples. Their precision is diminished by the presence of paramagnetic elements and other

mineral components. This liability impairs chemical characterization of whole soils and many of the SOM fractions that are recovered through physical extractions of soil.

Chemical extractions for SOM fractions are advantageous in this respect by providing purely organic fractions that are free of mineral components. Several different chemical extractants have been used to obtain distinct SOM fractions, which can contain substantial proportions of total SOM (Stevenson, 1994). However, chemical fractionations also have disadvantages. One concern is that chemical fractionations are often designed to maximize the quantity of extracted material to facilitate subsequent analyses for its chemical composition. The intent to extract as much material as possible could lead to coextraction of material from multiple SOM pools that differ in their chemical nature or function.

For example, NaOH is a popular chemical extractant because it generally extracts large quantities of humic material (Hayes et al., 1975; Stevenson, 1994; Swift, 1996). Extraction of humic substances by NaOH is yet more efficient when the soil is initially washed in an acid solution, normally HCl, before the NaOH extraction. The excess protons replace soil cations that bridge SOM to mineral surfaces and that provide internal binding within the organic macromolecule, stabilizing SOM against microbial degradation. The acid wash is most important for removal of polyvalent cations, especially Ca^{+2} (Mortensen, 1965; Stevenson, 1994; Swift, 1996), which are not completely removed from the soil by NaOH and are often the most abundant cations on soil mineral surfaces. Consequently, the acid wash generally results in much more efficient extraction of SOM, and it has become a common first step in extraction procedures involving NaOH.

Because the material extracted by NaOH after the acid wash was by definition chemically stabilized, it might conceivably differ from humic material that was not stabilized by polyvalent cations. The bound material may include SOM that cycles only slowly: recalcitrant SOM pools cycle over decades to centuries (Jenkinson, 1981), while N and other SOM-bound nutrients cycle on a daily basis. Consequently, the properties of the polyvalent cation-bound material may not fully capture the effects of recent crop management on SOM, and its analysis might not fully elucidate the causes of perturbed nutrient cycling. Yet, the initial step of acid wash results in coextraction of this material with SOM that is not stabilized by polyvalent cations and is conceivably more

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Abbreviations: CaHA, calcium humate; DAEHA, directly alkali-extractable humic acid; MHA, mobile humic acid; NMR, nuclear magnetic resonance; RMBHA, reducible metal-bound humic acid; SOM, soil organic matter.

likely to reflect the effects of recent crop management and to be involved in nutrient cycling.

If polyvalent cation-bound humic material were, however, extracted separately from unbound humic material, this distinction could be informative to nutrient cycling issues in a wide range of soils. Calcium is abundant, and its capacity to stabilize organic materials is well known, as reviewed by Baldock and Skjemstad (2000). Addition of Ca^{+2} reduced mineralization of glucose in a laboratory study and of straw under field conditions (Muneer and Oades, 1989a, 1989b). Decalcification of organo- Ca^{+2} complexes led to their dissociation and biodegradation in a laboratory study (Gaiffe et al., 1984). Levels of SOM were associated primarily with soil Ca in forest soils of eastern France (Tavant et al., 1994) and the Krakatoa islands of Indonesia (Higashi et al., 1987). Iron is also a well-known agent for stabilizing SOM (Baldock and Skjemstad, 2000), and other, less abundant polyvalent cations form even stronger bonds with SOM than do Ca^{+2} and Fe^{+3} (Schnitzer and Skinner, 1967; Finger and Klumberg, 1993). Addition of Ca^{+2} , Fe^{+3} , and other polyvalent cations reduced biodegradation of humates in laboratory studies (Juste et al., 1975).

A simple approach to distinguish SOM bound to polyvalent cations from unbound SOM has been to begin the fractionation procedure with a NaOH extraction, before the acid wash. The humic acid fraction that is recovered by this approach was named the MHA fraction in early work by Tyrurin (Kononova, 1966). Extraction of the MHA fraction can be followed by the acid wash to remove Ca^{+2} (Mortensen, 1965) and perhaps other polyvalent cations, then by a second NaOH extraction. The humic fraction that is recovered by the second NaOH extraction was named the CaHA fraction (Campbell et al., 1967). Both humic fractions are then normally cleansed of soil and salt contaminants, often through HF washes and dialyzing, respectively, and finally dried, typically through freeze-drying.

This report summarizes the few studies that applied the MHA and CaHA fractionation procedure toward gaining a process-level understanding of how crop management affects SOM properties, and how altered SOM properties in turn affect soil nutrient cycling and agroecosystem sustainability. This report considers specific criteria for demonstrating the relevance of a fractionation procedure to nutrient cycling. First, it should quantitatively provide fractions that have appreciable quantities for a wide range of soils. Their chemical natures should differ consistently across a wide range of

soils. Some of the fractions should be relatively labile: their quantities and chemical natures should be more responsive to crop management than are corresponding properties of total SOM. Finally, the effects of crop management on the quantities and chemical nature of humic fractions must affect nutrient cycling.

PROPERTIES OF THE MHA AND CaHA FRACTIONS

Cycling Rates Determined from Carbon Isotopes

In four studies involving ^{14}C dating of topsoil layers, the MHA fraction was dated as younger than the CaHA fraction, in three cases having modern ages or higher fraction Modern values that indicated rapid turnover (Table 1). A fraction Modern value > 100 implies the presence of ^{14}C produced by atmospheric weapons testing and hence a modern age that is too young to estimate. The CaHA fraction had older ^{14}C ages, or its fraction Modern values were slightly lower, indicating slower turnover. Campbell et al. (1967) found that the CaHA was considerably older than total SOM. The ^{14}C ages differed widely among the studies despite the use of similar extraction procedures, indicating an effect of soil type on humic properties.

Plants that follow the C3 pathway (Calvin cycle) discriminate against $^{13}\text{CO}_2$ during photosynthesis to a greater extent than do plants that follow the C4 pathway (Hatch-Slack cycle) (Deines, 1980). When the dominant vegetation on a soil is changed from C3 to C4 plants or vice versa, subsequent changes in the ratio of ^{13}C to ^{12}C , or the $\delta^{13}\text{C}$ value, can be determined for SOM fractions to determine their relative dynamics. Trends in the $\delta^{13}\text{C}$ values of the MHA and CaHA fractions for a Philippine rice soil with two annual crops indicated more rapid response of the MHA fraction than the CaHA fraction to crop changes. After five consecutive crops, the $\delta^{13}\text{C}$ value of the MHA differed by 2.3‰ between a double rice crop rotation and a rice-maize (*Zea mays* L.) rotation (Fig. 1), while the $\delta^{13}\text{C}$ value of the CaHA differed between the rotations by 1.1‰ (data not shown).

In Nebraska, assimilation rates of ^{13}C and ^{12}C from maize and soybean [*Glycine max* (L.) Merr.] residues indicated that the MHA fraction and total SOM consisted on average of younger material than did the CaHA fraction (Legorreta-Padilla, 2005). The light fraction, extracted from soil by flotation on a dense liquid of density 1.6 g cm^{-3} , had yet more dynamic changes than did the MHA fraction in contents of ^{13}C and ^{12}C following shifts

Table 1. ^{14}C dating of the mobile humic acid (MHA) fraction and calcium humate (CaHA) fraction that were extracted from surface layers.

Soil management	Soil properties			Mean residence time			Reference
	SOC†	Clay†	pH	MHA	CaHA	Whole soil	
	g kg ⁻¹ soil			yr			
Saskatchewan cultivated soil	56	—	—	785 ± 50	1410 ± 95	870 ± 50	Campbell et al. (1967)
California cotton soil	≈11	≈220	≈8.0	Modern	290	—	Olk et al. (1995)
Two Philippine rice soils	13.0 and 28.8	400 and 660	6.3 and 6.2	110‡ and 119‡	104‡ and 113‡	—	Olk et al. (1996)
Nebraska corn soil	≈20	≈430	≈6.0	≈10 to 20	546 ± 20	—	Legorreta-Padilla (2005)

† SOC is soil organic C or total soil C, and clay is soil clay content. Values for the California cotton soil and the Nebraska corn soil are approximate; exact values were not available for the specific soil depth (California) or soil sample (Nebraska).

‡ Fraction modern values. A value > 100 implies the presence of ^{14}C produced by atmospheric weapons testing and hence a modern age.

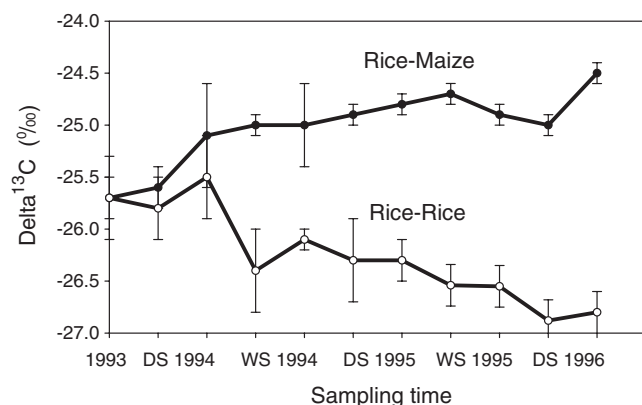


Fig. 1. Changes in the $\delta^{13}\text{C}$ value of the mobile humic acid fraction for the surface layer of a Philippine soil under two crop rotations. DS is dry season, WS is wet season. Soil samples were collected at the midtillering crop growth stage and at shortly before harvest in each season from 1994 to 1996. Vertical bars at each data point represent standard errors.

from C3 crops to C4 crops and also in assimilation of ^{15}N -labeled fertilizer N. The MHA gradually became more enriched in ^{15}N -labeled fertilizer N during a 1.5-yr span than did the light fraction, CaHA, or total SOM. Legorreta-Padilla (2005) concluded that the MHA and CaHA fractions provided a complementary approach to the light fraction, as the humic fractions cycled at slower rates than did the light fraction, and they provided more insights into N cycling while the light fraction was more insightful for C cycling.

Responsiveness of Humic Quantities to Land Use

In five studies representing a range of settings, the humic contents of C, expressed as g C kg^{-1} soil, were more responsive than total soil C to recent changes in land use (Table 2). The MHA contained 4 to 27% of total soil C in these studies, and the CaHA contained 8 to 15%. The sum of C content for both fractions was

generally 20–30% of total soil C. In all cases, the MHA-C content was highly responsive to land use, and in three cases the CaHA-C content was less responsive than the MHA-C content but more responsive than total soil C. One exception was a large increase in the CaHA-C content with increasing age of golf courses in Nebraska (Kerek et al., 2003). Soil exchangeable Ca also increased with golf course age, which may have caused increased stabilization of new SOM into the CaHA fraction. A second exception was the limited response of the CaHA-C content in a Nebraska soil to animal manure application (B. Eghball and D.C. Olk, 2005, unpublished data). The extraction procedure was nearly identical in all cases except the study by De Nobili et al. (1999), so this range of results indicates the large effect of soil type on humic properties.

In a study of Nebraskan long-term field experiments that ranged in duration from 4 to 18 yr, changes over time in C and N contents of whole soil and the MHA and CaHA fractions were significantly ($P < 0.1$) predicted by first-order kinetics in some of the field treatment combinations of fertilizer, crop rotation, and tillage (Legorreta-Padilla, 2005). For those treatment combinations with significant predictions, turnover times were calculated. The turnover time of total soil C ranged from 76 to 2500 yr and that of total soil N from 67 to 172 yr. Turnover times of MHA-C and MHA-N ranged from 8 to 35 yr and from 9 to 40 yr, respectively. The change over time in the CaHA-N content was not significantly predicted by first order kinetics in any treatment, and the change in CaHA-C content was significantly predicted solely for one treatment, and the turnover time was 21 yr. Hence, the MHA fraction cycled more rapidly than did total SOM for both C and N.

Chemical Properties

In several studies, the MHA and CaHA fractions were found to have consistently different chemical properties when analyzed by leading techniques for the chemical nature of SOM. In short, the MHA fraction was in-

Table 2. Effects of changes in land use on the relative increases in C content of the surface layer soil, the mobile humic (MHA) fraction, and the calcium humate (CaHA) fraction extracted from this soil.

Location	New land use and duration	Soil properties			Increase in C content \ddagger			% of SOC		Reference
		SOC \dagger	Clay \dagger	pH	Soil	MHA	CaHA	MHA	CaHA	
		g kg^{-1} soil			%					
Nebraska	Golf course construction, 4–28 yr	5–12	–	6.4–6.7	100	148	194	20–27	8–14	Kerek et al. (2003)
Nebraska	Animal manure application to low-C and high-C soils, 4 yr	7–32	–	5.7–7.6	64, 43	98, 64	43, 20	13–18	10–12	B. Eghball and D.C. Olk (2005, unpublished data)
Philippines	Increased number of annual lowland rice crops, 30 yr	13, 29	400, 660	6.3, 6.2	122	170	152	11–13	9–12	Olk et al. (1996)
Philippines	Increased fertilizer rates in continuous rice cropping at three sites, 25, 25, and 29 yr	14, 21, 22	460, 510, 610	6.4, 6.6, 6.1	12, 3, 13	76, 95, 36	21, 23, 3	5, 8, 11	14, 10, 9	Olk et al. (1998)
United Kingdom	Reversion of cultivated land to woodland and grassland, 113 and 153 yr	10, 42	–	–	265, 246	1491, 1739	533, 450	9, 11	9, 8	De Nobili et al. (1999)

\dagger SOC is soil organic C or total soil C, and clay is soil clay content.

\ddagger In case of >2 treatments, the increase was from the youngest to the oldest treatment or from least intensive to most intensive.

variably found to be composed of younger, more labile materials while the CaHA fraction was more humified; humification is defined as a gradual depletion of labile compounds such as amino compounds and carbohydrates and a gradual enrichment of unsubstituted aromatic rings and carboxyl groups (Stevenson, 1994; Zech et al., 1997).

The MHA fraction had substantially higher concentrations of N, S, and H compared with the CaHA fraction, providing for narrower ratios of C:N and C:S (Olk et al., 1999, 2000; Kerek et al., 2003). During humification, nitrogen pools are depleted through mineralization, and humification has been inversely correlated with H concentration (Yonebayashi and Hattori, 1988). These studies also found that the MHA fraction had lower concentrations of O, suggesting a lesser degree of microbially mediated oxidation. It had higher levels of amide N, diester P, and sugar diester P (Mahieu et al., 2002), which are considered as relatively labile nutrient forms. The MHA had lower levels of heterocyclic N and organic free radicals, and it absorbed less visible light; all trends indicating a lesser degree of humification (Mahieu et al., 2002; Senesi, 1990; Schnitzer and Lévesque, 1979). Analyses by ^{13}C cross polarization NMR spectroscopy found more prominent peaks in the CaHA than in the MHA for unsubstituted aromatics and carboxyl groups for a California cotton soil (Fig. 2) and Philippine rice soils (Olk et al., 1996, 1998), indicating a more humified state.

In rice soils, the MHA was highly enriched in phenolic lignin residues, as measured by ^{13}C cross polarization NMR and ultraviolet light absorption (Olk et al., 1996, 1998), saturation-pulse-induced dipolar exchange with recoupling—an NMR analysis for C forms bonded to N (Schmidt-Rohr et al., 2004)—and pyrolysis–gas chroma-

tography (Olk et al., 2002). Phenols were less abundant in the CaHA than in the MHA, but Olk et al. (2002) found the CaHA fraction was still more enriched with phenols than was total SOM. Phenolic lignin compounds are normally oxidized and degraded during humification (Flaig et al., 1975), so their enrichment represents an incomplete degree of decomposition and humification.

Changes in the intensity of lowland rice cropping affected the chemical properties of both fractions in a similar manner but to differing degrees. With increasing number of rice crops each year, the soil is flooded for longer durations and the amounts of incorporated crop residues are greater. Therefore, as the number of annual irrigated rice crops in field experiments increased from zero to three, less humified materials were observed to accumulate in SOM and both humic fractions, especially the MHA (Olk et al., 1996, 1998, 2000, 2002). A greater sensitivity of the MHA than the CaHA to cropping intensity was noted for elemental analysis and spectroscopic analysis by Fourier transform infrared spectroscopy (Olk et al., 1999, 2000), NMR analysis for ^{13}C , ^{15}N , and ^{31}P forms (Mahieu et al., 2002), and pyrolysis–gas chromatography (Olk et al., 2002). Visible light absorption at 465 nm, free radical concentrations, and H concentration, all easily measured indices of humification, were in most cases highly correlated with humic properties measured by ^{13}C , ^{15}N , and ^{31}P NMR for rice soils at three locations in the Philippines (Table 3). Field treatments at these sites included fertilizer rate and type (organic vs. inorganic, and for inorganic fertilizers none, +PK, or +NPK). The results indicate that (i) the MHA fraction was composed of more labile materials than was the CaHA fraction across a range of rice soils that differed in basic properties and crop management, and (ii) multiple chemical properties of the humic fractions were

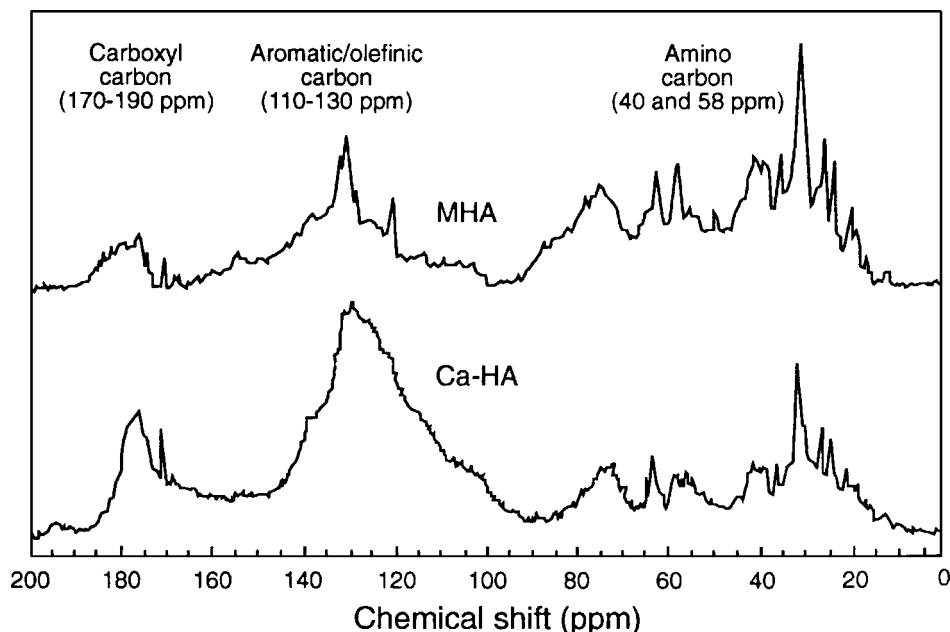


Fig. 2. Solid-state ^{13}C nuclear magnetic resonance spectra for the mobile humic acid (MHA) fraction and calcium humate (Ca-HA) fraction, extracted from the surface layer of a calcareous California soil planted to a cotton-based rotation. Reprinted from Olk et al. (1995) with permission from Elsevier Publishers.

Table 3. Correlation coefficients between spectral proportions from nuclear magnetic resonance analysis for N, C, and P functional groups with visible light absorption at 465 nm wavelength (E_{465}), free radical concentrations, and H concentration for the mobile humic acid fraction and the calcium humate fraction extracted from the surface layer of 17 Philippine rice soils. Soil organic C varied from 13.0 to 28.8 g kg⁻¹ soil (mean 19.2 ± 4.8 g kg⁻¹ soil), clay content varied from 400 to 660 g kg⁻¹ soil (mean 531 ± 81 g kg⁻¹ soil), and soil pH varied from 6.0 to 7.1 (mean 6.5 ± 0.3).†

Functional group	E_{465}	Free radicals	H concentration
N functional groups			
Heterocyclic	0.86***	0.85***	-0.86***
Amide	-0.81***	-0.78***	0.81***
Amine	0.17ns†	0.22ns	-0.13ns
Amino	0.45*	0.34ns	-0.50*
C functional groups			
N-alkyl	-0.94***	-0.84***	0.92***
O-alkyl	-0.41*	-0.48*	0.34ns
Acetals	-0.34ns	-0.42*	0.28ns
Aromatic	0.82***	0.81***	-0.80***
Phenolic	-0.70***	-0.69***	0.66***
Carboxylic	0.65***	0.61***	-0.64***
P functional groups			
Inorganic	0.58**	0.54**	-0.49*
Monoester	0.89***	0.78***	-0.88***
Sugar diester	-0.64***	-0.49*	0.68***
Diester	-0.75***	-0.71***	0.74***
Unknown	-0.18ns	-0.21ns	0.04ns
Monoester:diester	0.84***	0.75***	-0.84***

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

† Reproduced from Mahieu et al. (2002).

‡ ns, not significant.

responsive to crop management and location in consistent manners.

RELEVANCE OF THE MHA AND CaHA FRACTIONS TO NUTRIENT CYCLING

Few studies have attempted to link the quantity and chemical nature of the MHA and CaHA fractions to nutrient cycling processes in agroecosystems. Three case studies are reported below that (i) identified effects of recent crop management on the quantities and chemical nature of the MHA and CaHA fractions, and (ii) related these effects to nutrient cycling or the sustainability of the agroecosystems.

Soil Nitrogen Supply and Yield Decline in Intensive Irrigated Lowland Rice of Tropical Asia

A substantial portion of Asia's rice supply is produced by irrigated lowland rice farmers who grow two to three rice crops each year. Such intensive cropping is associated with frequent to nearly continuous soil submergence. The effects of submergence on soil properties and yield trends were evaluated in long-term field experiments of continuous double- and triple-cropped lowland rice. In those experiments in which initial grain yields approached the yield potential ceiling, the yields gradually declined during the subsequent 20 to 30 yr of continuous cropping (Cassman et al., 1995). Crop growth characteristics and plant N status indicated decreased

availability of soil N and a crop N deficiency during mid- to late-season growth stages. The quantity of total soil N in these field experiments had not decreased—and in fact it even increased—since these experiments were initiated. Therefore, research efforts were focused on possible changes in the quality of soil N, or its chemical forms, under long-term submerged conditions.

Lignin-derived phenolic compounds were shown to accumulate in the SOM of intensively cropped rice soils (Olk et al., 1996, 2002). In laboratory studies of model compounds, phenolic compounds covalently bind nitrogenous compounds into recalcitrant forms (Flaig et al., 1975). The resulting anilide-like structure is less plant-available than are the dominant soil N forms of amino acids and amino sugars (Verma et al., 1975; Stevenson and Cole, 1999), especially under the anoxic conditions of submerged soils. Chemical binding of N by phenolic lignin residues would be consistent with the observed decrease in availability of soil N even while the quantity of soil N did not decrease. Yet, until recently, no evidence for a substantial presence of anilide in SOM has been obtained for any field soil by any analysis, including cross polarization ¹⁵N NMR spectroscopy (Preston, 1996; Knicker et al., 1997).

The MHA fraction is well suited for studies of lignin–N interactions, given its enrichment in both N and lignin residues compared with total SOM, as described above. The MHA fraction from a triple-cropped rice soil in the Philippines (28.8 g total C kg⁻¹ soil, 660 g clay kg⁻¹ soil, soil pH 6.2) was analyzed for the chemical forms of C bonded to N by saturation-pulse-induced dipolar exchange with recoupling NMR spectroscopy (Schmidt-Rohr et al., 2004). Anilide structures were detected (Fig. 3a) and found to form from covalent binding of N by phenolic lignin residues. This MHA fraction had a substantially larger content of anilide (25% of all MHA-N) than did the MHA extracted from a nearby aerobic rice soil (11% of all MHA-N), and the magnitude of the difference between the two soils (55 kg N ha⁻¹) was of sufficient magnitude to potentially impact crop performance. The spectrum of the triple-cropped MHA fraction had a sufficiently prominent signal for aromatic N to enable estimation of the quantity of anilide N separate from that of heterocyclic N. These two compounds could not be distinguished in the spectrum of total SOM

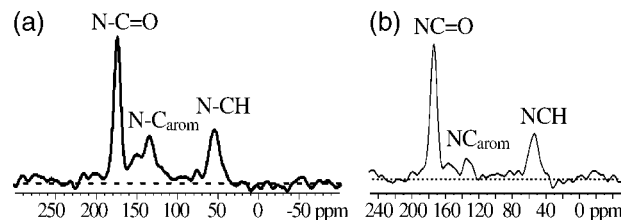


Fig. 3. Solid-state nuclear magnetic resonance spectra of ¹³C bonded to ¹⁴N in the (a) mobile humic acid fraction and (b) whole soil organic matter for the surface layer of a triple-cropped rice soil in the Philippines. The N–C=O signal is indicative of amides, the N–C_{arom} signal represents the aromatic N form anilide, and the N–CH signal is an aliphatic resonance of peptides. Figure 3a is reprinted from Schmidt-Rohr et al. (2004) with permission from the National Academy of Sciences of the USA. Figure 3b is from K. Schmidt-Rohr (2005, unpublished data).

(Fig. 3b) even after the mineral component of the soil was dissolved by HF washes: the anilide peak was much smaller for total SOM than for the MHA fraction.

Chemical analysis of the MHA fraction for rice soils from several long-term field experiments suggested that the phenol accumulation in rice soils—and hence the covalent binding of N—results primarily from anaerobic soil conditions during decomposition of incorporated crop residues (Olk et al., 1998). To test this hypothesis, anaerobic decomposition of crop residues was compared with aerobic decomposition in a field experiment of double-cropped rice in the Philippines (13–16 g total C kg⁻¹ soil, 560–570 g clay kg⁻¹ soil, soil pH 6.8–7.1). In the third year of the experiment, ¹⁵N-enriched urea fertilizer was applied at transplanting of the wet season crop. One week later, of all ¹⁵N that had been immobilized into the SOM, 29% was recovered in the MHA and 17% was recovered in the CaHA when averaged for both residue treatments, although each fraction accounted for only 9% of total soil N (Olk et al., 2006). By harvest, these ¹⁵N proportions for the MHA and CaHA fractions had decreased to 28 and 12%, respectively. The high rates of N immobilization and subsequent remineralization for the MHA and CaHA fractions indicate that both fractions were disproportionately involved in seasonal N cycling for this rice soil, especially the MHA fraction.

Of the ¹⁵N that was immobilized into the MHA fraction within 1 wk of fertilizer application, 45% was remineralized during the growing season in the aerobic decomposition treatment, while none was remineralized in the anaerobic decomposition treatment. This large difference between treatments was more subdued in the CaHA fraction: of the ¹⁵N that was immobilized into the CaHA fraction, 60% was remineralized in the aerobic decomposition treatment and 30% was remineralized in the anaerobic decomposition treatment. For total SOM, the corresponding decreases were 30 and 20%, respectively, indicating a yet smaller treatment difference. The effect of residue decomposition on ¹⁵N content at harvest for both this continuous rice rotation and also a rice–maize rotation was significant at $P = 0.02$ for the MHA fraction, $P = 0.05$ for the CaHA fraction, and $P = 0.37$ for total SOM. Compared with aerobic decomposition, anaerobic decomposition in the continuous rice rotation was associated with both a 22% reduction in crop uptake of soil organic N and also a phenol accumulation in the MHA fraction (data not shown). A phenol accumulation was not evident in the CaHA fraction. In summary, extraction of the MHA fraction enabled (i) more precise distinction of treatment effects on soil N cycling, and (ii) linkage of inhibited N mineralization with the phenol accumulation.

In a related study, kinetics of soil N mineralization were related to properties of whole soil, the MHA fraction, and the CaHA fraction for several rice soils of Vietnam and the Philippines (Nguyen et al., 2004). Soil organic C varied from 12.1 to 32.6 g kg⁻¹ soil (mean 18.7 ± 5.8 g kg⁻¹ soil), clay content varied from 150 to 680 g kg⁻¹ soil (mean 456 ± 156 g kg⁻¹ soil), and soil pH varied from 4.6 to 7.7 (mean 5.9 ± 0.9). Cumulative N mineralization during 84-d anaerobic incubations was

fitted to a two-pool first- and zero- order model. Three kinetic parameters that were calculated from the model were N_1 , the total size of a rapidly mineralizable N pool; k_1 , the rate constant for this rapidly mineralizable pool; and k_2 , the rate constant for a subsequent slower phase of steady state N mineralization. Of the three parameters, k_2 is perhaps the most relevant for predicting long-term soil N mineralization in field conditions. For all soils, the three parameters were predicted by stepwise regressions that included several properties of the soils and the MHA and CaHA fractions that had been extracted from these soils. Whole soil properties predicted 81% ($P < 0.001$), 27% (not significant at $P < 0.05$), and 36% ($P < 0.05$) of the variability in N_1 , k_1 , and k_2 , respectively. Inclusion of MHA properties into the stepwise regression analysis improved the prediction of variability in N_1 , k_1 , and k_2 to 96% ($P < 0.001$), 64% ($P < 0.05$), and 75% ($P < 0.01$), respectively. The MHA properties that contributed to the stepwise regression analysis were visible light absorption at 465 nm wavelength and C/N ratio. Inclusion of CaHA properties did not further improve the prediction of any kinetic parameter.

Cotton Lint Yield Decline and Potassium Deficiency in California

Cotton-based rotations are the dominant cropping system on the eastern side of the San Joaquin Valley, California. A long-term decline in cotton lint yield occurred during years of continuous cultivation because of a K deficiency (Cassman et al., 1989). Application rates of K fertilizer were not adequate to replace K lost in harvested crop biomass during the continuous cropping, and these vermiculitic soils developed a large fixation potential for K. The capacity of SOM to inhibit long-term mineral fixation of available K was demonstrated under field conditions by (i) an inverse relationship between SOM levels and mineral fixation of available K, and (ii) an increase in K availability with application of animal manure that was attributed to the organic component of the manure (Cassman et al., 1992).

To investigate the processes causing the benefit of SOM to K availability, a laboratory investigation was conducted using the MHA and CaHA fractions that were extracted from the calcareous Grangeville soil (Olk and Cassman, 1995). The MHA accounted for about 2% of total organic C in this soil, and the CaHA accounted for about 18%. Addition of both MHA and K to soil taken from the 0.20- to 0.40-m depth of the Grangeville soil (10.6 g organic C kg⁻¹ soil, 220 g clay kg⁻¹ soil, soil pH 8.1) resulted in greater 1 M NH₄-extractable K than with K addition alone during (i) a 7-d K adsorption isotherm (Fig. 4), (ii) three wetting/drying cycles to simulate moisture conditions of surface soil, and (iii) aerobic incubation for 84 d at two water potentials to simulate subsoil moisture conditions (data not shown). Addition of the MHA and K to the Nord soil (coarse-loamy, mixed, superactive, thermic Cumulic Haploxerolls; 8.1 g organic C kg⁻¹ soil, 180 g clay kg⁻¹ soil, soil pH 7.6) resulted in 42% more K uptake by sudangrass [*Sorghum × drummondii* (Steud.) Millsp. & Chase] after

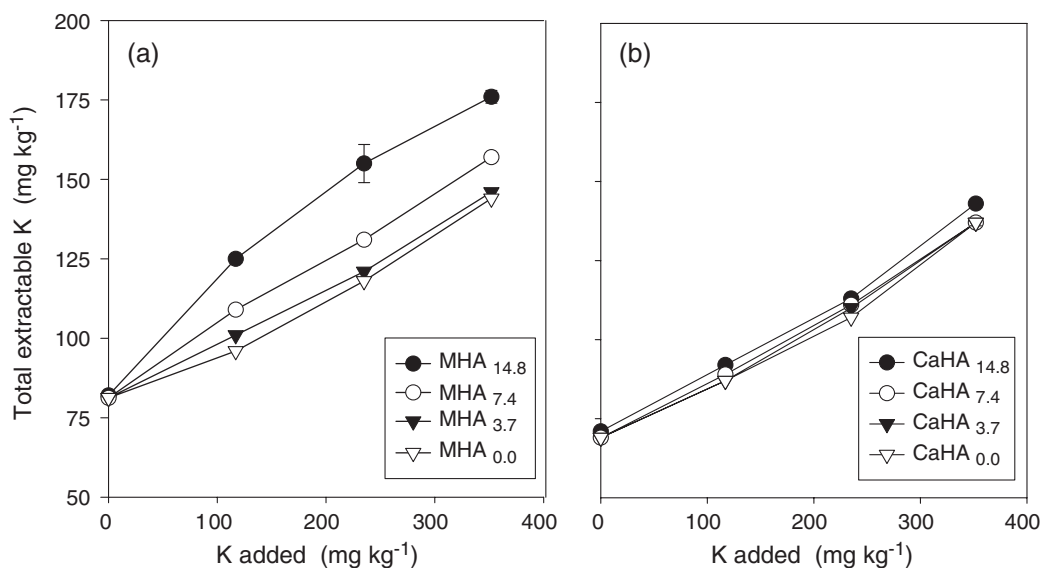


Fig. 4. Levels of 1 M NH₄-extractable K in the surface layer of a California cotton soil incubated for 7 d following addition of four K rates and the (a) mobile humic acid (MHA) fraction or (b) calcium humate (CaHA) fraction. The humic acid subscript is the amount of humic acid added (g kg⁻¹ soil). Standard error bars are smaller than the data points if not visible. Redrawn from Olk and Cassman (1995).

21 d growth compared with K addition alone. The increased K availability in these studies could not be explained by the small K content of the MHA, and so must have resulted from chemical interactions between the MHA and the soil. Addition of CaHA did not result in increased extractable K compared with K addition alone for either the K adsorption isotherm or the wetting-drying cycles. Hence, the increased K availability associated in field conditions with SOM content and animal manure application was reproduced in the laboratory solely by addition of the MHA fraction. The mechanism for increased K availability was shown to not involve dissolution of soil minerals or physical blockage of interlayer fixation sites. One possible explanation proposed by Olk and Cassman (1995) was exchange of interlayer K by amino groups of the N-rich MHA.

Rice Straw Management and Nutrient Cycling in Northern California

Burning was the preferred mode for disposal of rice straw in the Sacramento Valley of Northern California until air pollution concerns led to its banning. Subsequently, interest arose in incorporating the rice straw during winter flooding of the fields. To better understand the effects of straw incorporation and winter flood on soil N and C cycling and their ramifications for N fertilizer rates, Devêvre and Horwath (2001) extracted SOM fractions from the Maxwell rice soil (20.6 g total C kg⁻¹ soil, 510 g clay kg⁻¹ soil, soil pH 6.6) following a procedure related to the MHA/CaHA fractionation. The soil was first washed once by 0.1 M HCl to remove carbonates and floating debris. A subsequent NaOH extraction recovered the directly alkali-extractable humic acid (DAEHA) fraction. Then the soil was extracted repeatedly with Na₂S₂O₃ to remove polyvalent cations, including Fe, Mn, and Al. Finally, the reducible metal-bound

humic acid (RMBHA) fraction was extracted with NaOH. This procedure was based on the methods of McGill and Paul (1976), who measured greater amounts of recently added ¹⁵N in the DAEHA fraction than in the RMBHA fraction. The California soil had a near neutral pH, so the initial acid wash may have been partially or fully neutralized by the soil. Consequently, the DAEHA fraction might be comparable with the MHA fraction. The RMBHA fraction was presumed by the authors to have largely been Fe-bound, and as such it would be analogous to the CaHA fraction.

After adding ¹⁵N-labeled fertilizer N to the rice soil and then incubating it for 160 d either with or without straw addition under flooded or aerated conditions and at three temperatures, Devêvre and Horwath (2001) recovered at least four times as much ¹⁵N in the DAEHA fraction than in the RMBHA fraction for all treatments. Recovery of ¹⁵N in the DAEHA fraction increased significantly ($P < 0.05$) with straw incorporation, perhaps because straw incorporation stimulated microbial activity, facilitating biological immobilization (Devêvre and Horwath, 2001).

Bird et al. (2002) studied the distribution of ¹⁵N-labeled fertilizer N in a Maxwell soil (19.5 g total C kg⁻¹ soil, 510 g clay kg⁻¹ soil, soil pH 6.6) after a 2-yr field study of straw incorporation and winter flooding. They found that ¹⁵N-labeled fertilizer N had accumulated preferentially in the DAEHA fraction (named MHA) and the light fraction, compared with the RMBHA fraction (named metal-associated humic acid), humin, and a fulvic acid fraction that was recovered during DAEHA extraction. Half-lives of SOM-¹⁵N were calculated for both labile and stable components in each of the four chemically extracted fractions. For the stable component, the half-lives were 9 yr for the DAEHA, 7 yr for the mobile fulvic acid, 53 yr for the RMBHA, and 139 yr for the humin. In terms of ¹⁵N incorporation, Bird et al.

(2002) found that the DAEHA and light fraction were the most sensitive fractions to winter flooding and straw incorporation. These two fractions were also found to be the most interactive with shorter-term soil biology. The significance of the RMBHA fraction and humin was thought to lie more in long-term nutrient supply and sequestration of N and C.

DISCUSSION

Following the criteria listed above for demonstrating the relevance of a fractionation procedure to nutrient cycling, the MHA and CaHA fractionation appears to be a meaningful procedure in the limited number of studies described here. First, the MHA and CaHA fractions had appreciable quantities in most of the extracted soils, and these amounts were sensitive to soil type. The MHA and CaHA fractions are generally modest proportions of total SOM but appear to be relatively dynamic components. These results are consistent with previous observations that minority proportions of total SOM are highly involved in short-term nutrient cycling (Duxbury et al., 1989; Nicolardot et al., 1994).

Second, the MHA (and the equivalent DAEHA fraction) differed consistently from the CaHA (and the analogous RMBHA fraction) in relative age and chemical properties. The MHA was composed of younger materials than the CaHA fraction, as shown for several soils by ^{14}C -dating and for tropical rice soils by analyses for C, N, and P functional groups, H concentration, visible light absorption, and free radical concentrations. The MHA fraction cycled at faster rates than did the CaHA fraction, as shown by analyses for $\delta^{13}\text{C}$ values and ^{15}N cycling rates.

Third, compared with total SOM, the MHA fraction was relatively sensitive in its quantity and chemical nature to recent crop management, and MHA properties were more sensitive than were CaHA properties. In tropical rice soils, where this fractionation was extensively used, the CaHA fraction was more labile than total SOM in its quantity and degree of involvement in seasonal N cycling. In other agricultural soils, the lability of the CaHA fraction compared with that of total SOM was less consistent. Some evidence suggested that the CaHA may be more recalcitrant than was total SOM; for example, the ^{14}C ages reported by Campbell et al. (1967) as shown in Table 1 and also the smaller responses of CaHA quantity than total SOM quantity to animal manure application (Table 2) and to crop rotation (Legorreta-Padilla, 2005). Similar to other mechanisms for SOM stabilization, the significance of stabilization by Ca^{+2} and other polyvalent cations will vary with soil properties. Consequently, some variation in the lability of the CaHA is not surprising.

Finally, the quantities and biochemical nature of the MHA and CaHA provided novel insights into biochemical controls of nutrient cycling, especially when investigating issues of soil N cycling. The MHA fraction is enriched in N, and in the studies described here it was particularly involved in seasonal N cycling. Its enrichment in phenolic lignin residues compared to total SOM

in rice soils enabled linkage of its chemical nature to agronomically significant effects on N cycling. The lesser involvement of the CaHA fraction in N cycling and its smaller content of phenolic lignin residues compared with the MHA fraction (Olk et al., 2002) suggest it might better represent total SOM than does the MHA.

These insights would not have been possible if the MHA and CaHA had been coextracted as one fraction following the conventional initial step of acid washing. For example, the benefit of animal manure to plant-available K (Fig. 4) would not have been reproduced in laboratory studies by addition of humic material to the California soil if the MHA fraction had been coextracted with the much larger CaHA fraction.

This chemical fractionation enabled the distinction of labile SOM from recalcitrant SOM in flooded rice soils, but these results cannot be broadly extrapolated to aerobic upland soils because physical mechanisms for SOM stabilization may play a more prominent role in upland soils. Specifically, tropical flooded rice soils are puddled at the onset of each cropping season to destroy soil aggregation. Further, flooded rice soils are often finely textured and have less variation in particle sizes compared with many soils of upland cropping systems. In addition, the quantity of the MHA is likely to be enhanced in high-C soils like flooded rice soils, where C inputs are high and rates of SOM decomposition are slower than in aerobic soils. The general relevance of this fractionation procedure to nutrient cycling in soils can be more rigorously evaluated only after its further use in continuously aerated soils.

Data presented in this report and by Legorreta-Padilla (2005) indicate that the CaHA is not an inert fraction; its C and N fluxes occurred on a seasonal timescale. Therefore, this fraction is by itself not ideal for studying issues of long-term C stabilization such as C sequestration. Possible alternative options for chemically extracting inert C include (i) urea extraction of residual humic acids to isolate a purer humin fraction (Hayes, 2006, this issue), which is thought to consist of resistant, tightly bound aliphatic materials (Rice, 2001); and (ii) $\text{Na}_2\text{S}_2\text{O}_3$ or $\text{Na}_2\text{S}_2\text{O}_4$ extraction to solubilize oxides of Fe and perhaps Mn, Al, and Si, which can strongly stabilize SOM (Kiem and Kögel-Knabner, 2002).

Similar to other procedures for SOM fractionation, the MHA/CaHA fractionation can be a useful approach in specific situations, but it is not without problems. First, it is laborious: typically at most six soils can be extracted per week, and this schedule requires full-time labor. Second, any distinction between the two fractions appears lost in highly acidic soils, for example agroforestry soils from Costa Rica that have pH values of 3.4 to 4.7 (2004, unpublished data). Third, quantities of the MHA are often extremely small in low-C soils. Nevertheless, MHA properties contributed to elucidating nutrient cycling in low-C soils for application of animal manure to California cotton soils (Olk and Cassman, 1995) and amendment of soybean N to a tropical vegetable soil (Thönnissen et al., 2000). A final potential problem is that as a chemical fractionation, this procedure cannot capture the effects on SOM dynamics of

the spatial arrangement of primary and secondary organomineral particles in soil.

Recommendations

Recommendations for future action include standardization of the acid wash step to ensure consistent extraction of the CaHA fraction. Acid molarity, shaking duration, and number of acid washes can affect the degree to which polyvalent cations are removed and consequently the efficacy of the subsequent alkaline extraction for the CaHA. For example, when two calcareous Iowa soils were washed repeatedly with 0.2 M HCl until the pH of the acid supernatant remained below 1.0, the quantity of the CaHA fraction that was subsequently extracted was two- and five-fold greater than when the acid wash was stopped at a pH endpoint of 1.3 (2004, unpublished data). A general guideline for exhaustive removal of polyvalent cations from all soils should be based on the final pH of the acid wash or a negative test for polyvalent cations in the acid wash. The tremendous variation among soils in their contents of exchangeable cations and carbonates precludes the probability that a prespecified number of acid washes will exhaustively yet efficiently remove polyvalent cations from all soils.

A comparison is needed of the different techniques used to remove soil polyvalent cations: acid wash principally for Ca^{+2} , $\text{Na}_2\text{S}_2\text{O}_3$ or $\text{Na}_2\text{S}_2\text{O}_4$ extraction principally for Fe^{+3} and Al^{+3} (Devêvre and Horwath, 2001; McGill and Paul, 1976; Kiem and Kögel-Knabner, 2002), and $\text{Na}_4\text{P}_2\text{O}_7$ for direct extraction of humic material bound to some polyvalent cations (Schnitzer, 1982). It is unknown whether the humic fractions that are extracted subsequently to these three steps have the same chemical natures. If they differ consistently, the most suitable soil types and research issues for each technique should be identified.

Finally, the significance of cationic stabilization to cycling of SOM and nutrients will vary greatly by soil type and research issue. Chemical, biological, and physical mechanisms for SOM stabilization operate in all soils to varying degrees. A comprehensive strategy for a range of soil types and research issues would integrate the MHA/CaHA fractionation with other fractionation approaches, whether as independent fractionations of separate subsamples or as successive steps in one integrated procedure. Bird et al. (2002) and Legorreta-Padilla (2005) demonstrated the synergistic benefits of extracting the light fraction in addition to the MHA and CaHA, indicating that labile SOM can be distinguished from recalcitrant SOM in one soil by both physical and chemical means.

CONCLUSIONS

Distinction of the MHA and CaHA fractions is based on the known stabilization of SOM by polyvalent cations, especially Ca^{+2} . This fractionation appears to be a potentially useful approach for studying nutrient cycling. The quantities of both fractions were apprecia-

ble though not dominant proportions of SOM in most soils. In most cases, both fractions were more responsive to land use changes than was total SOM in quantity and chemical nature, and the MHA fraction was clearly more responsive than the CaHA fraction. The MHA fraction was found to be composed of more labile materials than was the CaHA fraction in terms of their chemical constituents and cycling rates of C and N. Finally, in the limited number of studies that used this fractionation to understand the role of SOM in nutrient cycling, treatment effects on the content and chemical nature of the MHA fraction were linked to nutrient cycling trends, while the CaHA fraction provided less consistent results. The high contents of N and phenolic lignin residues in the MHA fraction enabled its application to studying chemical interactions between soil N and phenolic compounds.

Given the limited number of studies that have applied this fractionation to nutrient cycling issues, it needs further evaluation on a greater range of soil types, especially in aerobic soils. It could also be combined with biological or physical fractionations for yet better distinction of labile SOM from recalcitrant SOM.

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